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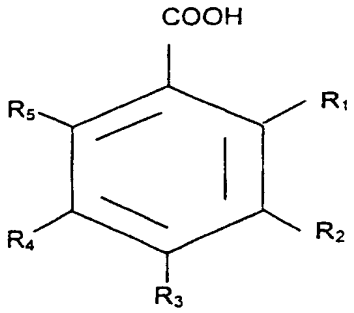

(1)

(57) Abstract

The present invention consists in a method for inducing stress tolerance in plant material, the method comprising the step of applying to plant material an effective stress–regulating amount of one or more active compounds of formula (1) or a functional derivative thereof, wherein R_1 to R_5 are selected from the group consisting of hydrogen, loweralkyl, oxo, amino, carbonyl, halogen, thio, phosphate, sulfoxide, sulfone, deuterium, carboxyl, aldehyde, hydroxy, hydroxyloweralkyl, alkoxyloweralkyl, loweralkoxycarbonyl, loweracyloxyloweralkyl, actylloweralkyl, loweralkanoyl, loweralkylamino, diloweralkylamino, loweralkoxy, loweracyloxy, loweralkylthio, loweralkyl sulphonyl, loweralkyl sulphinyl, or cycloalkyl or cycloalkoxy having from 4 to 6 carbon atoms which is optionally substituted by loweralkyl, halogen, oxygen, hydroxy or loweralkoxy, such that the selection of R_1 to R_5 results in a compound capable of inducing stress tolerance in plant material.

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Method for Inducing Stress Tolerance in Plant Material

Technical Field

The present invention relates to a method for inducing stress tolerance in plant material through the use of active compounds such as benzoic acid, salicylic acid or a functional derivative thereof.

Background

Billions of dollars are lost annually throughout the world by the agricultural and horticultural industry as a result of over exposure of plants to adverse environmental conditions. Such conditions cause stress in plants reducing their resistance to environmental conditions and or causing them to become susceptible to attack by fungus and other diseases. Over time various methods have been advanced to protect plants and plant products from stress injury, including those caused by chilling, freezing, heat, drought and salt.

Plant life is susceptible to damage from stress associated with variations in temperature and moisture, injurious chemicals and combinations, and biological attack. Flowers, leaves, and other portions from plants that are cut also rapidly lose their fresh appearance due to the stress caused by such cutting. Substantial efforts have been made to extend the resistance of plants to stress associated with temperature and other causes as well as lengthening the shelf life of flowers, leaves and other portions from plants.

U.S. Patent No. 2,805,137 discloses a process for conditioning cut flowers by applying an effective amount of a composition comprising a phenol, a compound selected from the group consisting of carbonyl-containing compounds and compounds capable of being catabolized by plant enzymes to carbonyl-containing compounds and a compound selected from the group consisting of hydrazines, amines and quaternary ammonium compounds. Among the hydrazines and amines, which are alleged to have antiseptic properties and the ability to lower the surface tension of water and therefore suited for use are mono and diamines such as methyl-amine, ethylamine, diamines such as

ethylene diamine, trimethylenediamine, and polyamines such as triamino methane, tetramethylene-tetramine and hexamethylene-tetramine. The phenol is present in a proportion by weight of 5-1200 parts, the carbonyl-containing compound from 5,000 to 50,000 parts and the hydrazine or amine at a level from 10-900 parts.

U.S. Patent No. 4,231,789 discloses the application of long chain polyamine compounds of the general formula H_2N - R_1-NH_2 wherein R_1 represents a -$(CH_2)_n$- where n represents an integer from 4-18 and various polyethylene polyamine amino derivatives thereof as a method for protecting crops from suffering various damages due to temperature, etc. Examples of suitable amines within the formula include tetramethylenediamine, pentamethylenediamine, hexadecamethylene-diamine, spermidine and other polyamines. The polyamine is diluted to a concentration generally in the range of 10^{-4} to 10^{-2} moles per liter and applied as an aqueous formulation.

While a variety of compounds have been used in an attempt to control the adverse effects of environmental stresses on plants, the compounds presently available suffer from a number of drawbacks. For example, the triazole compounds (paclabutrazol, uniconzole, triadimephon), are used either to treat seeds or soil. Although it has been demonstrated that triazoles may induce stress tolerance, the compounds have side effects. The most notable side effect being growth retardation (stunting) caused by the inhibition of gibberellin biosynthesis.

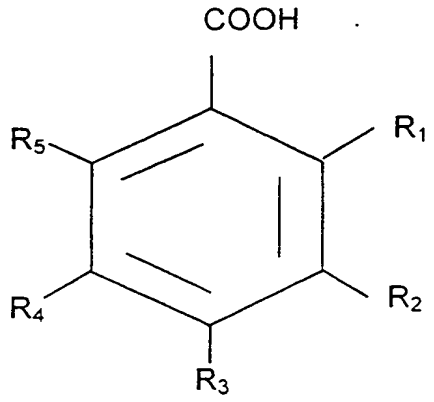
The present invention seeks to provide a method for inducing and or increasing tolerance to stress in plant material. At the very least the present invention seeks to provide an alternative to the methods and compounds that are presently available.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers including method steps.

Description of the Invention

The present invention consists in a method for inducing stress tolerance in plant material, the method comprising the step of applying to plant material an effective stress-regulating amount of one or more compounds of the following formula (1) or functional derivative(s) thereof:

Formula 1



wherein R_1, to R_5 are selected from the group consisting of hydrogen, loweralkyl, oxo, amino, carbonyl, halogen, thio, phosphate, sulfoxide, sulfone, deuterium, carboxyl, aldehyde, hydroxy, hydroxyloweralkyl, alkoxyloweralkyl, loweralkoxycarbonyl, loweracyloxyloweralkyl, actylloweralkyl, loweralkanoyl, loweralkylamino, diloweralkylamino, loweralkoxy, loweracyloxy, loweralkylthio, loweralkyl sulphonyl, loweralkyl sulphinyl, or cycloalkyl or cycloalkoxy having from 4 to 6 carbon atoms which is optionally substituted by loweralkyl, halogen, oxygen, hydroxy or loweralkoxy, such that the selection of R_1, to R_5 result in a compound capable of inducing stress tolerance in plant material. Desirably the selected compound is capable of being converted to or degraded to benzoic acid or salicylic acid following application to the plant material.

In one preferred form of the invention R_3, and R_5 are both hydrogen while R_1, R_2 and R_4 are selected from the group consisting of hydrogen, loweralkyl, oxo, amino, carbonyl, halogen, thio, phosphate, sulfoxide, sulfone, deuterium, carboxyl, aldehyde, hydroxy, hydroxyloweralkyl, alkoxyloweralkyl, loweralkoxycarbonyl, loweracyloxyloweralkyl, actylloweralkyl, loweralkanoyl, loweralkylamino, diloweralkylamino, loweralkoxy, loweracyloxy, loweralkylthio,

loweralkyl sulphonyl, loweralkyl sulphinyl, or cycloalkyl or cycloalkoxy having from 4 to 6 carbon atoms which is optionally substituted by loweralkyl, halogen, oxygen, hydroxy or loweralkoxy.

In a highly preferred form of the invention R_1 is hydrogen, a hydroxy or an acetyloxy group, R_2 is hydrogen or a lower alkyl group and R_3 is hydrogen or a sulfoxide group. Desirably, the active compound is selected from the group consisting of Benzoic acid, 2-Hydroxy 5-sulfobenzoic acid (Sulfosalicylic acid), 2-Hydroxy Benzoic acid (Salicylic Acid), 2-Hydroxy 3-methylbenzoic acid (Methyl Salicylic acid) or 2-Acetyloxy Benzoic Acid (Acetyl Salicylic acid).

A functional derivative of an active compound is any derivative that is adapted to or capable of inducing stress tolerance. Preferably, functional derivatives include any compound that may be converted or degraded to benzoic acid or salicylic acid.

The compounds employed in the present invention (hereinafter referred to as active compounds) may also be employed as esters or salts thereof and for purposes of this invention the derivative salt form is equivalent to and incorporated into the terminology benzoic acid equivalents or salicylic acid equivalents. The conversion of naturally occurring compounds to salts for application to plants is known and that technology is applicable to the utilisation of the active compounds described here. Examples of such salts include potassium benzoate, sodium benzoate and the like.

Preparation of the above compounds may be achieved by any method known in the art. In this respect most of the above compounds are known in the art and may be prepared by well-practiced prior art methods.

Active compounds employed in the method of the present invention may be applied to various forms of plant material including, for example, whole plants such as seedlings and portions thereof such as cuttings, plant tissues or organs (*in vitro* and *ex vitro*) and cells, protoplasts, fruit, flowers, seeds and microspore cultures. Preferably the method is applied to un-harvested whole plants.

When applying the fore mentioned active compounds, the effective stress-regulating amount of active compound delivered to a plant material should be sufficient to exert or effect desired protection of the plant material to the stress that may be anticipated. Such effective stress-regulating amounts will vary not only with the particular active compound selected for application, but also with the level of induced stress tolerance to be achieved, the species of plant being treated and its stage of development, and whether a permanent or transient regulating effect is sought. Other factors which may bear upon the determination of an effective stress-regulating amount of active compound include, for example, the plant growth medium, the manner in which the treatment is to be applied, weather conditions such as temperature or rainfall, the amount and quantity of water absorbed by the plant material, the concentration of the compound in solution, the surface area of the plant material exposed and the ease of transfer of the compound to the interior of the plant cells.

With regard to the specific activity of the active compound it should be understood that some compounds may be far more active than other compounds. For example, benzoic acid has been found to be particularly active at much lower concentrations than salicylic acid. Field tests have also demonstrated that the effects of the active compounds may also vary from one plant species to another depending on the nature and the concentration of the compound used. Hence, some of the active compounds may be highly specific to certain plant species while others may not.

An effective stress-regulating amount of the active compounds should serve to induce stress tolerance in the plant material. Preferably, an effective stress-regulating amount of active compounds is a concentration of between approximately 0.001mM and 1.0 mM. More preferably, an effective stress-regulating amount of active compounds is a concentration of between approximately 0.05mM and 0.75 mM. Desirably, the active compound concentration is between approximately 0.01mM and 0.5mM. More specifically the active compound concentration is between approximately 0.1mM and 0.5mM.

The stresses to which the present invention may induce tolerance may be varied. Preferably, the stress tolerance is one or more of the stress tolerances in the group comprising: desiccation tolerance, drought tolerance, temperature tolerance such as freezing tolerance and heat tolerance, salinity tolerance, post transplant stress tolerance, post harvest weight loss, post harvest multiple stress tolerance and herbicide tolerance. More particularly, the method of the present invention may be used to reduce and preferably prevent plant death (whole or part of) or growth inhibition of seed derived, somatic embryo derived or micropropagated (tissue/cell culture derived) plants or plants derived from cuttings, which are exposed to stresses after transplanting and prior to complete plant establishment, as well as after establishment.

In accordance with the present invention it has been found that induction of stress tolerance may be achieved by application of the active compounds to plants in various stages of development. Accordingly, in the practice of this invention the active ingredient can be applied to the soil habitat of the plant or directly to the plant in the seedling stage, flowering stage or fruiting stage and the like or can be applied sequentially to plants at more than one stage of development. Such application may be made directly to one or more of the plant's parts, such as stems, leaves, flowers, fruit, seeds or the like. Generally, the application is made by spraying or drenching or imbibing seeds or by irrigation of the plants using conventional techniques.

In a particularly preferred form of the invention the active compounds are applied throughout the life of a plant as a hardening method to prevent injury or death by exposure to stress at any time of the plant's life cycle. For example, when applied to plants prior to harvest or to harvested plant material such as fruit the method of the present invention may enhance the post harvest shelf life of the plant material (stems, leaves, fruits etc). The method of the present invention may also be used to enhance cryopreservation and cold storage ability of plant material such as plant tissues. Further, seeds may be imbibed in one or more of the active compounds sowed or dried prior to planting.

Applicant believes that other hardening treatments such as heat shock or chilling treatment when used in conjunction with the active compounds enhance the stress tolerance induced. Therefore, in one embodiment of the invention there is provided a method for inducing stress tolerance in plant material, the method comprising the steps of: (i) subjecting a plant to heat shock or chilling treatment; and (ii) applying to a plant material an effective stress-regulating amount of benzoic acid or a functional derivative thereof.

Active compounds may be applied as a single compound (diluted to appropriate levels with a suitable dilution agent) or as a plurality of active compounds. Where a plurality of active compounds are employed they may be applied individually or in combination. For example, salicylic acid and acetyl salicylic acid may be formulated to be released in combination or alternatively they may be formulated to be released independently of each other. If formulated for independent release either salicylic acid or acetyl salicylic acid may be prepared to be released immediately or shortly after application to plant material while the other chemical may be formulated in a slow release applicator to ensure continual release of the other active compound over a long period of time.

The rate of application will depend on a number of factors, such as the amount of stress that the plant is likely to be subjected to or is subjected to, environmental conditions, type of plant species and the like.

The timing of the application and rate of application appear to bear a relationship to one another and to the plant species to which the compounds are applied, such that the rate of application and the timing thereof bear a relationship to the result observed.

Application of the described compounds can be achieved by a variety of processes and means. Preferably the compounds are applied in combination with one or more application vehicles. When delivered in such a manner the prepared composition must contain and be capable of releasing an effective stress tolerant amount of an active compound to exert or effect desired protection of the plant to the stress that may be anticipated. Preferably, the active compounds are applied to the plant material with at least one formulation

auxiliary selected from the following groups: solid carriers; solvents or dispersing agents; surfactants (wetting agents and emulsifiers); dispersants (without surfactant action); and stabilisers. Using auxiliaries of these types and others, the active compounds can be prepared into customary formulations, such as dusts, powders, granules, solutions, emulsions, suspensions, emulsifiable concentrates, pastes and the like.

Where the active compounds are water-insoluble they can be formulated following methods customary for water-insoluble compounds, using the known formulation auxiliaries. The compositions can be prepared in a manner known per se, for example by mixing the particular active substance with solid carriers, by dissolving or suspending in suitable solvents or dispersing agents, if desired with the use of surfactants as wetting agents or emulsifiers and/or disperstants, or by diluting of already prepared emulsifiable concentrates with solvents or dispersing agents.

Usually the active compounds of this invention will be applied in the form of a concentrate that can be readily extended with an inert carrier prior to application to the plants. Concentrates in solid form are, for example, water soluble powders consisting of finely divided solids such as calcium silicate, surfactant and from about 1-95% or more by weight of the active ingredient which are diluted with water prior to applying to the plants.

In one form of the invention, the active compounds may be applied as an aqueous solution to the plant surface to permit absorption of the compounds into the plant. When applied in such a manner the concentration of active compound in the formulation may vary widely, e.g. from 0.0001 to 99%. Desirably the active compounds when applied in liquid form are mixed with a solvent, surfactant, emulsifier, defoamer and/or additive and about 0.0001 to 95% by weight of the formulation is active ingredient. More preferably, the concentration of active compound in the formulation will vary from about 0.0001 to 70%. In a highly preferred form of the invention, the concentration of active compound in the formulation will vary from about 0.0001 to 50% and more specifically

between about 0.0001 and 25%. In a particular example the concentration will vary between 0.0001 and 1.0%.

The aqueous solution may also be prepared as a liquid concentrate that can be diluted with water to provide a composition suitable for application to plants. Some of these active compounds are active at very low dosages and therefore their ability to inhibit stress should not be confused with the herbicidal properties that have been observed when some of the compounds are applied at higher concentrations.

In an alternative form of the invention the active compounds may be applied to the plant material impregnated on finely divided or granular inorganic or organic carriers such as attapulgite clay, sand vermiculite, corn cobs, activated carbon or other granular carriers known to the art. The impregnated granules may then be spread on the soil.

In an another form of the invention the active compounds may be formulated, for example, as a wettable powder by impregnating an inactive powdered carrier to which a surface-active dispersing agent has been added.

To prepare preparations in the form of powders, the active compounds can be mixed with a solid carrier, for example by grinding them together. Alternatively, the solid carrier can be impregnated with a solution or suspension of the active substance, and the solvent or dispersing agent is then removed by evaporation, heating or filtering off under reduced pressure. Such compositions in the form of powders can be rendered readily wettable with water by adding surfactants or dispersants, so that they can be converted into aqueous suspensions which are suitable, for example, as compositions for spraying. The wettable powder may then be dispersed in water and sprayed on plants, or the soil surface, or plants to be prepared for harvesting. Similarly, an emulsifiable concentrate may be prepared by dissolving the active agents in a suitable solvent to which a surface-active dispersing agent has been added. The emulsifiable concentrate may then be dispersed in water and applied by spraying.

The following are mainly suitable as solid carriers: natural minerals, such as chalk, dolomite, limstone, clays and silica and their salts, for example kieselguhr, kaolin, bentonite, talc, attapulgite or montmorillonite; synthetic minerals such as highly disperse silica, aluminia or silicates; organic substances such as cellulose, starch, urea or synthetic resins; and fertilisers such as phosphates or nitrates. Such carriers can be, for example, in the form of powders or granules.

The following are mainly suitable as solvents or dispersing agents: aromatic substances such as benzene, toluene, xylene and alkylnaphthalenes; chlorinated aromatic substances and chlorinated aliphatic hydrocarbons such as chlorobenzene, chloroethylenes or methylene chloride; aliphatic hydrocarbons such as cyclohexane or parafins, for example mineral oil fractions; alcohols such as butanol or glycol and their ethers and esters; ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone; and strongly polar solvents or dispersing agents, such as dimethylformamide, N-methylpyrrolidone or dimethyl sulfoxide (solvents of these types preferably have flash points of at least 30°C and boiling points of at least 50°C), or water. Other suitable solvents or dispersing agents are also so-called liquefied gaseous extenders or carriers, which are products that are gaseous at room temperature and under atmospheric pressure. Examples of such products are, in particular, aerosol propellants such as halohydrocarbons, for example dichlorodifluoromethane.

The surfactants (wetting agents and emulsifiers) can be non-ionic compounds such as: condensation products of fatty acids, fatty alcohols or fat-substituted phenols with ethylene oxide; fatty acid esters and fatty acid ethers of sugars or polyhydric alcohols; the products which are obtained from sugars or polyhydric alcohols by condensation with ethylene oxide; block polymers of ethylene oxide and propylene oxide; or alkyldimethylamine oxides. The surfactants can also be anionic compounds such as: soaps; fatty sulfate esters, for example sodium dodecyl sulfate, sodium octadecyl sulfate or sodium cetyl sulfate; alkylsulfonates, arylsulfonates or fatty aromatic sulfonates, such as alkylbenzenes sulfonates, for example calcium dodecylbenzenesulfonate or butylnaphthalenesulfonates; or more complex fatty sulfonates, for example the amide condensation products of oleic acid and N-methyltaurine, or the sodium

sulfonate of dioctyl succinate. Finally, the surfactants can be cationic compounds such as alkyldimethylbenzylammonium chlorides, dialkyldimethylammonium chlorides, alkylmethylammonium chlorides or ethoxylated ammonium chlorides.

Suitable dispersants (without surfactant action) are mainly the following: lignin, sodium slats and ammonium salts of ligninsulfonic acids, sodium salts and ammonium salts of sulfonated polycondensation products from naphthalene and formaldehyde, or sulfite waste liquors. Examples which can be employed as dispersants which are particularly suitable as thickeners or sedimentation inhibitors are methylcellulose, carboxymethylcellulose, hydroxyethylcellulose, polyvinyl alcohol, alginates, caseinates or blood albumin.

Examples of suitable stabilisers are: acid-binding agents, for example epichlorohydrin, phenyl glycidyl ethers or soya epoxides; antioxidants, for example gallic esters or butylhydroxytoluene; UV absorbers, for example substituted benzophenones, diphenylacrylonitrilic esters or cinnamic esters; or deactivators, for example salts of ethylenediaminotetraacetic acid, or polyglycols.

Chemical compositions in addition to the fore mentioned active compounds may also be applied to the plant material in conjunction with the active compounds. Such compounds may be mixed with or included in, for example, insecticides, acaricides, nematicides, molluscicides, bactericides, fungicides, herbicides, plant growth regulators, fertilisers and trace element sources. Such combined compositions are suitable for broadening the spectrum of action.

Examples

Features of the present invention are more fully described in the following Examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the invention, and should not be understood in any way as a restriction on the broad description as set out above.

Example 1

Benzoic acid

Garden bean (cv. Garden Beauty), and tomato (cv Romano) plants were grown as a test species in pots (135 mL volume) in potting soil mixture in a glasshouse with ambient temperature. Seventeen day old seedlings were treated (soil drenched) with 20 mL of 0.1, 0.25 mM benzoic acid (BZA), and plants were exposed to stress 8 days and 21 days after treatment.

Seeds were also imbibed in the above solutions for 24 h and planted as above and plants were exposed to stress 21 days after planting.

Plants were chilled at 1°C in a growth chamber for 2 days respectively to simulate chilling injury. All the benzoic acid treated plants at appropriate concentrations of BZA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 1 & 2).

Plants were grown and water was withheld for 6 days. All the benzoic acid treated plants at appropriate concentrations of BZA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 1 & 2).

Plants were exposed to 50°C heat for 2.5 h in an oven with light. All the benzoic acid treated plants at appropriate concentrations of BZA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 1 & 2).

Bean and tomato leaves were sprayed with 1mL of benzoic acid solution at varying solutions. The respective plants were then subjected to chilling, heat and drought in accordance with the above methods. All the benzoic acid treated plants at appropriate concentrations of BZA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation,

chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 3 & 4).

Bean and tomato seedlings were treated with benzoic acid and the pots were saturated with 200 mM NaCl for beans and 800 mM for tomato. Treated plants survived and did not display any injury 3 days after the treatment. Control plants were wilted (See Table 5).

Bean seedlings were sprayed with 500 ul per litre Gramoxone 250™, a commercial herbicide (active ingredient is paraquat dichloride). Control plants displayed necrotic lesions typical of paraquat injury within 24 h. Treated plants did not display any injury symptoms at this time (see table 6).

Table 1: Bean plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solution. Plants were exposed to stress at specified days after benzoic acid treatment.

BZA	Chilling (% survival)[1]			Heat (% survival)[1]			Drought (% survival)[1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.25	100	100	100	100	100	100	100	100	100
0.5	50	60	60	50	60	60	50	65	65

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 2: Tomato plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after benzoic acid treatment.

BZA	Chilling (% survival)[1]			Heat (% survival)[1]			Drought (% survival)[1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.25	100	100	100	100	100	100	100	100	100
0.5	50	60	65	60	70	55	60	65	45

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 3: Bean plants were sprayed with 1mL of benzoic acid solution at varying concentrations. The plants were then subjected to chilling, heat and drought in accordance with the above methods

BZA Conc (mM)	Chilling Survival (%)[1]	Heat Survival (%)[1]
0	0	0
0.1	95	100
0.5	90	95

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 4: Tomato plants were sprayed with 1mL of benzoic acid solution at varying solutions. The plants were then subjected to chilling, heat and drought in accordance with the above methods

BZA Conc (mM)	Chilling Survival (%)[1]	Heat Survival (%)[1]
0	0	0
0.1	100	100
0.5	60	55

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 5: Salt treatment: Plants were treated with 200mM (bean) and 800mM (tomato) NaCl. Pots were saturated with salt solution and observations were made.

BZA	Bean (% of plants, day 3)			Tomato (% of plants, day 3)		
(mM)	Wilted	Necrosis	Survival[1]	Wilted	Necrosis	Survival[1]
0	100	100	0	100	100	0
0.1	0	0	100	0	0	100

Note: Control plant showed chlorosis and stunted growth on day 8.

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 6: Gramaxone treatment: 8 days after soil drenching with BZA, the plants were sprayed using a hand held sprayer, with gramaxone (500µL/L). Each plant received 1mL of herbicide solution. Damaged leaf area was estimated as a percentage of total.

BZA Conc	Bean Leaf Damage (% area)		Tomato Leaf Damage (% area)	
(mM)	day 1	day 2	day 1	day 2
0	25	50	20	60
0.1	0	5	0	5
0.25	0	10	0	10
0.5	30	60	40	60

Bean plants were treated with benzoic acid at 0.1 mM concentration 10 days before harvesting and the weight loss was monitored. The treated pods lost 25% less weight over 6 days at room temperature.

Solute (electrolyte) leakage: 10 leaf discs from bean and tomato plants were taken and incubated in 5ml of deionized water and the conductivity of the solution was measured using a conductivity meter. The tubes containing leaf discs were heated in a water bath at 95°C for 5 min to kill all cells. The conductivity of the solution was recorded as a total and is illustrated in table 7.

Seedlings of *Eucalyptus marginata* and geranium (cv Elite Red) were treated with benzoic acid and petiole sections were harvested and cultured on sterile nutrition media containing growth regulators auxins and cytokinins and thidiazuron and monitored the tissue browning. Substantial reduction in tissue browning and death during excision and in culture was observed in benzoic acid treated plants.

Table 7: Solute (electrolyte) leakage: 10 leaf discs were taken and incubated in 5mL of deionized water and the conductivity of the solution was measured using a conductivity meter. The tubes containing leaf discs were heated in a water bath at 95°C for 5 minutes to kill all cells. The conductivity of this solution was recorded as total.

BZA (mM)	Bean Conductivity (% of total)	Tomato Conductivity (% of total)
0	16	18
0.1	7	5

Example 2

Sulfosalicylic acid

Garden bean (cv. Garden Beauty), and tomato (cv Romano) plants were grown as a test species in pots (135 mL volume) in potting soil mixture in a glasshouse with ambient temperature. Seventeen day old seedlings were treated (soil drenched) with 20 mL of 0.1, 0.25 and 0.5 mM 2-hydroxy 5-sulfobenzoic acid (SSA), and plants were exposed to stress 8 days and 21 days after treatment.

Seeds were also imbibed in the above solutions for 24 h and planted as above and plants were exposed to stress 21 days after planting.

Plants were chilled at 1°C in a growth chamber for 2 days respectively to simulate chilling injury. All the 2-hydroxy 5-sulfobenzoic acid treated plants at appropriate concentrations of SSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 8 & 9).

Plants were grown and water was withheld for 6 days. All the 2-hydroxy 5-sulfobenzoic acid treated plants at appropriate concentrations of SSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 8 & 9).

Plants were exposed to 50°C heat for 2.5 h in an oven with light. All the 2-hydroxy 5-sulfobenzoic acid treated plants at appropriate concentrations of SSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 8 & 9).

Bean and tomato seedlings were treated with 2-hydroxy 5 sulfobenzoic acid and the pots were saturated with 200mM NaCl for beans and 800mM for tomato. Treated plants survived and did not display any injury 3 days after the treatment. Control plants were wilted (See Table 10).

Bean seedlings were sprayed with 500 ul per litre Gramoxone 250™, a commercial herbicide (active ingredient is paraquat dichloride). Control plants displayed necrotic lesions typical of paraquat injury within 24 h. Treated plants did not display any injury symptoms at this time (See Table 11).

Seedlings of *Eucalyptus marginata* and geranium (cv Elite Red) were treated with 2-hydroxy 5-sulfobenzoic acid and petiole sections were harvested and cultured on sterile nutrition media containing growth regulators auxins and cytokinins and thidiazuron and monitored the tissue browning. Substantial reduction in tissue browning and death during excision and in culture was observed in benzoic acid and 2-hydroxy 5-sulfobenzoic acid treated plants.

Table 8: Bean plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after Sulfosalicylic acid (SSA) treatment.

SSA	Chilling (% survival)[1]			Heat (% survival)[1]			Drought (% survival)[1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.25	100	100	100	100	100	100	100	100	100
0.5	90	100	100	100	100	100	100	100	100

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 9: Tomato plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after Sulfosalicylic acid (SSA) treatment.

SSA	Chilling (% survival)[1]			Heat (% survival)[1]			Drought (% survival)[1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.25	100	100	100	100	100	100	100	100	100
0.5	90	100	100	100	100	100	100	100	100

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 10: Salt treatment: Plants were treated with 200mM (bean) and 800mM (tomato) NaCl. Pots were saturated with salt solution and observations were made after 3 days.

SSA	Bean (% plants, day 3)			Tomato (% plants, day 3)		
(mM)	Wilted	Necrosis	Survival[1]	Wilted	Necrosis	Survival[1]
0	100	100	0	100	100	0
0.1	0	0	100	0	0	100
0.25	0	0	100	0	0	100
0.5	0	0	100	0	0	100

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 11: Gramaxone treatment: 8 days after soil drenching with SSA, the plants were sprayed using a hand held sprayer, with gramaxone (500µL/L). Each plant received 1mL of herbicide solution. Damaged leaf area was estimated as a percentage of total.

SSA Conc	Bean Leaf Damage (% area)		Tomato Leaf Damage (% area)	
(mM)	day 1	day 2	day 1	day 2
0	30	60	20	50
0.1	0	10	0	5
0.25	0	10	0	10
0.5	0	10	0	10

Example 3

Salicylic acid

Bean (cv. Garden Beauty), corn and tomato (cv Romano) plants were grown as a test species in pots (135 ml volume) in potting soil mixture in a glasshouse with ambient temperature. Seventeen day old seedlings were treated (soil drenched) with 20 mL of 0.1, 0.5 mM salicylic acid (SA)(salicylic acid was obtained from Sigma™, cat. no. S-7401) and plants were exposed to stress 3 days and 8 days after the treatment.

Seeds were also imbibed in the above solutions for 24 h and planted as above and plants were exposed to stress 21 days after planting.

Plants were chilled at 4°C for 9 days without watering. All the salicylic acid treated plants at appropriate concentrations of SA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 12 to 14).

Plants were grown and water was withheld for 6 days. All the salicylic acid treated plants at appropriate concentrations of SA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 12 to 14).

Plants were exposed to 50°C heat for 2.5 h in an oven with light. All the salicylic acid treated plants at appropriate concentrations of SA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 12 to 14).

Beans plants were sprayed with 1mL of salicylic acid solution at varying solutions. The respective plants were then subjected to chilling, heat and drought in accordance with the above methods. All the salicylic acid treated plants at appropriate concentrations of SA survived whereas all the control

plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 15).

Bean seedlings were treated with salicylic acid and the pots were saturated with 200 mM NaCl. Treated plants survived and did not display any injury. Control plants displayed salt injury 24 h after the treatment and died 8 days after salt treatment.

Bean seedlings were sprayed with 500 ul per litre Gramoxone 250™, a commercial herbicide (active ingredient is paraquat dichloride). Control plants displayed necrotic lesions typical of paraquat injury within 24 h. Treated plants did not display any injury symptoms.

Seedlings of *Eucalyptus marginata* and geranium (cv Elite Red) were treated with salicylic acid and petiole sections were harvested and cultured on sterile nutrition media containing growth regulators auxins and cytokinins and thidiazuron and monitored the tissue browning. Substantial reduction in tissue browning and death during excision and in culture was observed in salicylic acid treated plants.

Table 12: Bean plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after salicylic acid treatment.

SA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 13: Tomato plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after salicylic acid treatment.

SA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	Day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 14: Corn plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after salicylic acid treatment.

SA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 15: Bean plants were sprayed with 1mL of salicylic acid solution at varying solutions. The plants were then subjected to chilling, heat and drought in accordance with the above methods

SA Conc	Chilling (% survival) [1]		Heat (% survival) [1]		Drought (% survival) [1]	
(mM)	day 8	day 21	day 8	day 21	day 8	day 21
0	0	0	0	0	0	0
0.5	100	100	100	100	100	100

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Example 4

Acetyl salicylic acid

Bean (cv. Garden Beauty), corn (cv. Honeysweet) and tomato (cv Romano) plants were grown as a test species in pots (135-ml volume) in potting soil mixture in a glasshouse with ambient temperature. Seventeen day old seedlings were treated (soil drenched) with 20 mL of 0.1, 0.5 mM acetyl salicylic acid (ASA) (acetyl salicylic acid was obtained from Sigma™, cat. no. A-5376) and plants were exposed to stress 3 days and 8 days after the treatment.

Seeds were also imbibed in the above solutions for 24 h and planted as above and plants were exposed to stress 21 days after planting.

Plants were chilled at 4°C for 9 days without watering. All the acetyl salicylic acid treated plants at appropriate concentrations of ASA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 16 to 18).

Plants were grown and water was withheld for 6 days. All the acetyl salicylic acid treated plants at appropriate concentrations of ASA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 16 to 18).

Plants were exposed to 50°C heat for 2.5 h in an oven with light. All the acetyl salicylic acid treated plants at appropriate concentrations of ASA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Tables 16 to 18).

Beans plants were sprayed with 1mL of acetyl salicylic acid solution at varying concentrations. The respective plants were then subjected to chilling, heat and drought in accordance with the above methods. All the acetyl salicylic acid treated plants at appropriate concentrations of ASA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation,

chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Table 19).

Table 16: Bean plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after acetyl salicylic acid treatment.

ASA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 17: Tomato plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after acetyl salicylic acid treatment.

ASA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 18: Corn plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after ASA treatment.

ASA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 3	day 8	day 21	day 3	day 8	day 21	day 3	day 8
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 19: Bean leaves were sprayed with 1mL of acetyl salicylic acid solution at varying solutions. The plants were then subjected to chilling, heat and drought in accordance with the above methods

ASA Conc	Chilling (% survival) [1]		Heat (% survival) [1]		Drought (% survival) [1]	
(mM)	day 8	day 21	day 8	day 21	day 8	day 21
0	0	0	0	0	0	0
0.5	100	100	100	100	100	100

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

The plant height of acetyl salicylic acid treated and untreated plants (soil drenched on 14th day) were not significantly different (means were 18.5 and 18.8 cm respectively on the 21st day after planting) (see Tables 20 & 21)

Bean seedlings were treated with acetyl salicylic acid and the pots were saturated with 200 mM NaCl. Treated plants survived and did not display any injury. Control plants displayed salt injury 24 h after the treatment and died 8 days after salt treatment.

Bean seedlings were sprayed with 500 uL per litre Gramoxone 250™, a commercial herbicide (active ingredient is paraquat dichloride). Control plants displayed necrotic lesions typical of paraquat injury within 24 h. Treated plants did not display any injury symptoms.

Solute (electrolyte) leakage: 10 leaf discs from bean and tomato plants were taken and incubated in 5mL of deionized water and the conductivity of the solution was measured using a conductivity meter. The tubes containing leaf discs were heated in a water bath at 95C for 5 min to kill all cells. The conductivity of the solution was recorded as a total and is illustrated in Table 22. Treated leave discs exhibited lower solute leakage than untreated plants indicating that the cellular integrity of the ASA treated leaf discs was greater than untreated leaf discs.

Table 20: Height of 14 day old bean plants, grown from seeds imbibed in acetyl salicylic acid solution.

Imbibition Treatment (mM ASA)	Mean Height (cm ± Standard Deviation)
0	22.1 ± 1.8
0.01	21.5 ± 2.0
0.05	22.6 ± 1.6
0.10	21.9 ± 1.4
0.25	21.7 ± 1.0
0.50	22.2 ± 2.2

Table 21: Height of 21 day old bean plants soil drenched with acetyl salicylic acid at 14 days of age.

ASA Concentration (mM)	Mean Height (cm ± Standard Deviation)
0	18.8 ± 1.0
0.5	18.5 ± 1.2

Table 22: Solute (electrolyte) leakage: 10 leaf discs were taken and incubated in 5mL of deionized water for 30 minutes and the conductivity of the solution was measured using a conductivity meter. The tubes containing leaf discs were heated in a water bath at 95°C for 5 minutes to kill all cells. The conductivity of this solution was recorded as total.

ASA Conc (mM)	Bean Conductivity (% of total)	Tomato Conductivity (% of total)
0	13	16
0.5	5	6

Microsomal membranes were isolated from bean leaves, 7 days after acetyl salicylic acid treatment (soil drench), and the lipid transition temperature of the membrane was measured using differential scanning calorimetry (see Table 23).

Seedlings of *Eucalyptus marginata* (jarrah) and geranium (cv Elite Red) were treated with acetyl salicylic acid and petiole sections were harvested and cultured on sterile nutrition media containing growth regulators auxins and cytokinins and thidiazuron and monitored the tissue browning. Substantial reduction in tissue browning and death during excision and in culture was observed in acetyl salicylic acid treated plants (see Tables 24 & 25).

Table 23: Differential Scanning Calorimetry: microsomal membranes were isolated from bean leaves, 7 days after ASA treatment (soil drench), and the lipid transition temperature of the membrane was measured using differential scanning calorimetry.

ASA Concentration (mM)	Bulk Phase Transition (°C) of Microsomal Membranes of Bean Leaves
0	-7.4
0.5	-14.2

Table 24: *Eucalyptus marginata* seeds were sterilized and germinated *in vitro* in the dark for 2 weeks and seedlings were incubated in 0.5 mM solution of ASA for 3 days. The leaf petioles were excised and 1cm sections were cultivated on media containing 1/2 MS nutrients (Murashige, T. and Skoog, F., (1962) Physiologia Plantarum, 15:473-499), vitamins and 10μM Thidiazuron. Tissue browning was monitored.

ASA Conc (mM)	7 days after culture	21 days after culture
0	100% very light brown	30% light brown 30% dark brown 40% yellowish brown
0.5	100% yellowish green	50% yellowish green 50% very light brown

Table 25: Geranium (cv Elite Red) was sprayed with 0.5 mM ASA. One week later leaf petioles were harvested, sterilized and cultured on media containing 1/2 MS nutrients (Murashige, T. and Skoog, F., (1962) Physiologia Plantarum, 15:473-499), vitamins and 10μM Thidiazuron.
The tissue browning was visually estimated using these classifications.
0 - no browning
1 - less than 10% of the area has brown colour and no dark leachate around the tissue
2 - 10 to 50% of the tissue is brown with dark leachate around the tissue
3 - greater than 50% of the tissue is brown in colour with surrounding dark leachate.

ASA Conc (mM)	7 days after culture	21 days after culture
0	1	3
0.5	0	1

Example 5

Methyl salicylic acid

Bean (cv. Garden Beauty), tomato (cv Romano) and corn (cv. Honeysweet) plants were grown as a test species in pots (135-ml volume) in potting soil mixture in a glasshouse with ambient temperature. Seventeen day old seedlings were treated (soil drenched) with 20 ml of 0.1, 0.5 mM methyl salicylic acid (MSA) and plants were exposed to stress 3 days and 8 days after the treatment.

Seeds were also imbibed in the above solutions for 24 h and planted as above and plants were exposed to stress 21 days after planting.

Plants were chilled at 4°C for 9 days without watering. All the methyl salicylic acid treated plants at appropriate concentrations of MSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 26 to 28).

Plants were grown and water was withheld for 6 days. All the methyl salicylic acid treated plants at appropriate concentrations of MSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 26 to 28).

Plants were exposed to 50°C heat for 2.5 h in an oven with light. All the methyl salicylic acid treated at appropriate concentrations of MSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation, chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (See Tables 28 to 28).

Beans plants were sprayed with 1mL of methyl salicylic acid solution at varying concentrations. The respective plants were then subjected to chilling, heat and drought in accordance with the above methods. All the methyl salicylic acid treated plants at appropriate concentrations of MSA survived whereas all the control plants exhibited severe leaf and growing point injury (wilting, desiccation,

chlorosis, necrosis in whole or part) often resulting in a failure of plants to maintain normal growth (see Table 29).

Table 26: Bean plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after methyl salicylic acid treatment.

MSA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 27: Tomato plants were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after methyl salicylic acid treatment.

MSA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	Day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

1: Survival defined as the ability of the plant to maintain growth habits during and after stress treatment

Table 28: Corn plants (cv. Honeysweet) were grown from seeds imbibed (SI) in solutions for 24 hours or 17 day old plants were soil drenched (SD) with 20mL of solutions. Plants were exposed to stress at specified days after methyl salicylic acid treatment.

MSA	Chilling (% survival) [1]			Heat (% survival) [1]			Drought (% survival) [1]		
Conc	SI	SD		SI	SD		SI	SD	
(mM)	day 21	Day 8	day 21	day 21	day 8	day 21	day 21	day 8	day 21
0	0	0	0	0	0	0	0	0	0
0.1	100	100	100	100	100	100	100	100	100
0.5	100	100	100	100	100	100	100	100	100
1.0	0	0	0	0	0	0	0	0	0

Note: SI, Seed Imbibed; SD, Soil Drenched

Table 29: Bean plants (cv garden bounty)were sprayed with 1mL of methyl salicylic acid solution at varying solutions. The plants were then subjected to chilling, heat and drought in accordance with the described methods.

MSA Conc	Chilling (% survival) [1]		Heat (% survival) [1]		Drought (% survival) [1]	
(mM)	day 8	day 21	day 8	day 21	day 8	day 21
0	0	0	0	0	0	0
0.5	100	100	100	100	100	100

Tuart (*Eucalyptus gomphocephala*) leaves were sprayed with varying concentrations of methyl salicylic acid and then subjected to heating at 58C for 2.5 hours. Application of MSA and ASA (at 0.5mM) substantially halted injury to heat treated leaves (see Table 30)

A branch containing leaves of the tree *Corymbia ficifolia* (*Eucalyptus ficifolia*) of *Eucalyptus* and geranium (cv Elite Red) were treated with methyl salicylic acid and petiole sections were harvested and cultured on sterile nutrition media containing growth regulators auxins and cytokinins and thidiazuron and monitored the tissue browning. Substantial reduction in tissue browning and death during excision and in culture was observed in acetyl salicylic acid treated plants (see Tables 31 & 32).

Therefore, agricultural and horticultural compositions comprising as an active ingredient an effective stress-regulating amount of at least one of the above mentioned compounds may be very useful for treating agricultural, forestry and horticultural plants which may be exposed to state of stresses, for example, in salty regions such as on reclaimed land, coast land or a region closed by a desert susceptible to be damaged by a drought, or in regions where sudden chills or excessive heat are prevalent or where transplant trauma may be significant in reducing plant vigour or survival.

Table 30: Tuart (*Eucalyptus gomphocephala*) leaves were sprayed with varying concentrations of methyl salicylic acid and then subjected to heating at 58°C for 2.5 hours

Concentration (Spray treatment)	Percentage of injured leaves after heating at 58°C for 2.5 hours (%)
Water	100
0.1 mM MSA	40
0.25 mM MSA	36
0.5 mM MSA	6
0.5 mM ASA	14

Note: ASA used for comparison.

Table 31: Branch of leaves of Corymbia ficifolia (*Eucalyptus ficifolia)* tree was sprayed with 0.5 mM MSA. One week after spraying the leaf petioles were sterilized and 1 cm sections were cultured on agar medium containing 1/2 MS nutrients (Murashige, T. and Skoog, F., (1962) Physiologia Plantarum, 15:473-499), vitamins and growth regulators, 0.5 µM Benzylaminopurine. Tissue browning and survival was monitored.

MSA Conc (mM)	7 days after culture	28 days after culture
0	Dead, dark brown	Dead
0.5	All alive, yellowish green	75% alive, light brown

Table 32: Geranium (cv Elite Red) was sprayed with 0.5 mM MSA. One week later leaf petioles were harvested, sterilized and cultured on media containing 1/2 MS (Murashige, T. and Skoog, F., (1962) Physiologia Plantarum, 15:473-499), vitamins and 10μM Thidiazuron.

The tissue browning was visually estimated using these classifications.

0 - no browning

1 - less than 10% of the area has brown colour and no dark leachate around the tissue

2 - 10 to 50% of the tissue is brown with dark leachate around the tissue

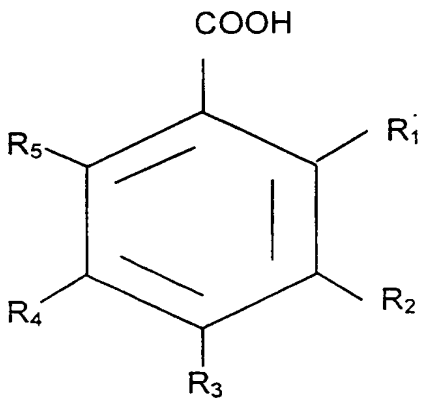
3 - greater than 50% of the tissue is brown in colour with surrounding dark leachate.

MSA Conc (mM)	7 days after culture	21 days after culture
0	1	3
0.5	0	1

The Claims

1. A method for inducing stress tolerance in plant material, the method comprising the step of applying to a plant material an effective stress-regulating amount of one or more active compounds of the following formula (1) or a functional derivative thereof:

Formula 1



wherein R_1, to R_5 are selected from the group consisting of hydrogen, loweralkyl, oxo, amino, carbonyl, halogen, thio, phosphate, sulfoxide, sulfone, deuterium, carboxyl, aldehyde, hydroxy, hydroxyloweralkyl, alkoxyloweralkyl, loweralkoxycarbonyl, loweracyloxyloweralkyl, actylloweralkyl, loweralkanoyl, loweralkylamino, diloweralkylamino, loweralkoxy, loweracyloxy, loweralkylthio, loweralkyl sulphonyl, loweralkyl sulphinyl, or cycloalkyl or cycloalkoxy having from 4 to 6 carbon atoms which is optionally substituted by loweralkyl, halogen, oxygen, hydroxy or loweralkoxy, such that the selection of R_1, to R_5 result in a compound capable of inducing stress tolerance in plant material.

2. A method according to claim 1 wherein R_3, and R_5 are both hydrogen while R_1, R_2 and R_4 are selected from the group consisting of hydrogen, loweralkyl, oxo, amino, carbonyl, halogen, thio, phosphate, sulfoxide, sulfone, deuterium, carboxyl, aldehyde, hydroxy, hydroxyloweralkyl, alkoxyloweralkyl, loweralkoxycarbonyl, loweracyloxyloweralkyl, actylloweralkyl, loweralkanoyl, loweralkylamino, diloweralkylamino, loweralkoxy, loweracyloxy, loweralkylthio, loweralkyl sulphonyl, loweralkyl

sulphinyl, or cycloalkyl or cycloalkoxy having from 4 to 6 carbon atoms which is optionally substituted by loweralkyl, halogen, oxygen, hydroxy or loweralkoxy.

3. A method according to claim 1 wherein R_1 is hydrogen, a hydroxy or a acetyloxy group, R_2 is hydrogen or a lower alkyl group, R_3 is hydrogen, R_4 is hydrogen or a sulfoxide group and R_5 is hydrogen.

4. A method according to claim 1 wherein the active compound is selected from the group consisting of Benzoic acid, 2-Hydroxy 5-sulfobenzoic acid, 2-Hydroxy Benzoic acid, 2-Hydroxy 3-methylbenzoic acid, or 2-Acetyloxy Benzoic Acid.

5. A method according to claim 4 wherein the active compound is salicylic acid or a functional derivative thereof.

6. A method according to claim 1 wherein the plant material is selected from the group consisting of: whole plants or cuttings, plant tissues and organs or cells, protoplasts, fruit, flowers, seeds or microspore cultures.

7. A method according to claim 1 wherein the plant material is un-harvested whole plants.

8. A method according to claim 1 wherein the effective stress-regulating amount of active compound delivered to a plant material is a concentration of between approximately 0.001mM and 1.0mM.

9. A method according to claim 1 wherein the effective stress-regulating amount of active compound delivered to a plant material is a concentration of between approximately 0.05mM and 0.75mM.

10. A method according to claim 1 wherein the effective stress-regulating amount of active compound delivered to a plant material is a concentration of between approximately 0.01mM and 0.5mM.

11. A method according to claim 1 wherein the effective stress-regulating amount of active compound delivered to a plant material is a concentration of between approximately 0.1mM and 0.5mM.

12. A method according to claim 1 wherein the active compounds are applied to a plant during different stages of its growth.

13. A method according to claim 1 wherein the active compounds are applied to soil, to the soil habitat of a plant or directly to the plant in the seedling stage, flowering stage or fruiting stage.

14. A method according to claim 12 wherein the active compounds are applied directly to one or more of the plant's parts.

15. A method according to claim 1 wherein the active compounds are applied by one or more of the following methods: spraying, drenching, seed imbibing or by irrigation of the plants.

16. A method according to claim 1 wherein the active compounds are applied to enhance cryopreservation and cold storage ability of plant material such as plant tissues.

17. A method according to claim 1 wherein a plurality of active compounds are applied to the plant material.

18. A method according to claim 16 wherein the active compounds are formulated for independent release of each other.

19. A method according to claim 16 wherein one of the active compounds is prepared to be released immediately or shortly after application to the plant material while the other compound is formulated in a slow release applicator which permits release of the other active compound over a long period of time.

20. A method according to claim 1 wherein the active compounds are applied in combination with one or more application vehicles.

21. A method according to claim 19 wherein the application vehicles are selected from the following groups: solid carriers; solvents or dispersing agents; surfactants (wetting agents and emulsifiers); dispersants (without surfactant action); and stabilisers.

22. A method according to claim 1 wherein the active compounds are prepared in a form selected from the following group: dusts, powders, granules, solutions, emulsions, suspensions, emulsifiable concentrates or pastes.

23. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 to 99% by weight of the formulation.

24. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 to 95% by weight of the formulation.

25. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 to 70% by weight of the formulation.

26. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 to 50% by weight of the formulation.

27. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 and 25% by weight of the formulation.

28. A method according to claim 1 wherein the active compounds are applied as an aqueous solution in a concentration of from 0.0001 and 1.0% by weight of the formulation.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00949

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A01N 37/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A01N 37/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: IPC and (plant: or tree: or veget: or agricult: or horticult:)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 52370/96 A (SST AUSTRALIA PTY LTD) 5 December 1996	1-28
Y	Derwent Abstract Accession No. 93-150079/18, Class C01, SU 1732901 A (A MED PLANTS PHYSIOLOGY BIOCHEM INST) 15 May 1992	1-28

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 January 1999

Date of mailing of the international search report
2 FEB 1999

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00949

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
AU	52370/96	NZ	286629	US	5693592

END OF ANNEX